J. Clin. Chem. Clin. Biochem.
Vol. 21, 1983, pp. 695–702

The Influence of the Label on the Quality of a Solid-Phase Immunoassay: Evaluation of a Commercial ELISA Kit for Serum Ferritin

By *H. A. Assink, H. J. Brouwer, B. G. Blijenberg* and *B. Leijnse*

Department of Clinical Chemistry, University Hospital Rotterdam-Dijkzigt and Department of Chemical Pathology, Erasmus University, Rotterdam, The Netherlands

(Received February 11/June 6, 1983)

Summary: A new Enzyme Linked Immuno Sorbent Assay (ELISA) kit for the determination of serum ferritin has been compared with another ferritin kit based on the Immuno Radio-Metric Assay (IRMA) approach, both assays containing similar antibodies.

Based on these studies, we found the within-run precision of the ELISA (and IRMA) to have coefficients of variation of 4–10% and 2–6% respectively, over a concentration range of 12–600 μg/l. The between-run precision for the same concentration range exhibited a CV range of 9–13% and 7–11% respectively. The sensitivities were found to be 1.4 μg/l and 0.9 μg/l. The mean recovery was 103% for the ELISA procedure.

It was found that, using the serum dilution technique, the linearity reached to 1000 μg/l.

In the ELISA procedure no influence from the so-called "high dose hook effect" was observed. While EDTA-plasma produced 6% lower values than serum in the ELISA technique, no interference from albumin, γ-globulins and mild haemolysis was observed. Stability problems with the ELISA kit were not encountered. A comparative analysis of multiple specimens demonstrated nearly identical values with $r = 0.994$ and $y = 0.87\ x^{1.01}$.

The quality and ease of operation of the ELISA approach compared with other techniques are discussed.

In conclusion it is possible to replace a radio-label in an immunoassay with an enzyme-label with the same degree of reliability and other parameters of quality control exhibited by radioimmunoassays.

Einfluß der Markers auf die Qualität eines Festphasen-Immunassay: Prüfung eines käuflichen ELISA-Bestecks für Ferritin im Serum

Zusammenfassung: Ein neues Enzymimmunoassay(ELISA)-Besteck für die Bestimmung von Ferritin im Serum wurde mit einem Ferritin-Besteck nach dem Prinzip des immunradiometrischen Assay (IRMA) verglichen; beide Bestecks enthielten ähnliche Antikörper.

Für die Präzision in der Serie fanden wir für einen Konzentrationsbereich von 12–600 μg/l Variationskoeffizienten von 4–10% (ELISA) und 2–6% (IRMA), für die Präzision von Tag zu Tag 9–13% bzw. 7–11%. Die Empfindlichkeit war 1,4 bzw. 0,9 μg/l. Die mittlere Wiederfindung betrug für das ELISA-Verfahren 103%. Die Linearität untersucht durch Verdünnungstechnik reichte bis 1000 μg/l. Bei dem ELISA-Verfahren wurde kein Einfluß durch den sog. „high dose hook effect" beobachtet. Während EDTA-Plasma mit dem ELISA-Verfahren 6% niedrigere Werte als Serum ergab, bestand kein Einfluß von Albumin, γ-Globulinen oder geringe Hämolyse.

Haltbarkeitsprobleme mit dem ELISA-Besteck ergaben sich nicht. Die Vergleichsanalyse an einer Vielzahl von Proben ergab nahezu identische Werte mit $r = 0{,}994$ und $y = 0{,}87\ x^{1.01}$. Die Qualität und Arbeitsweise des Enzymimmunassays wird im Vergleich mit anderen Verfahren diskutiert.

Zusammenfassend stellen wir fest, daß es möglich ist, die Radiomarkierung im Immunassay mit dem selben Grad an Zuverlässigkeit und anderer Qualitätskontroll-Kenngrößen von Radioimmunassays durch eine Enzymmarkierung zu ersetzen.

Introduction

Ferritin is the major storage protein of iron in the body. It is located mainly in the reticuloendothelial system and is found predominantly in the liver, spleen and bone marrow. The mean serum ferritin concentration correlates quite well with the total body iron store in clinical conditions ranging from iron deficiency to iron overload. Increased serum ferritin levels can also be found in patients with some types of malignancy, liver disease and inflammation (1–4).

The first immunoassay sensitive enough to detect ferritin in normal serum was developed by *Addison* et al. in 1972 (5). In their assay, they employed an immunosorbent and ^{125}I-labeled anti-ferritin antibody and termed the technique an immunoradiometric assay (IRMA) to distinguish it from typical radioimmunoassays. Within 2 years, *Miles* et al. introduced an improved and simpler assay, which they designated as a "two-side IRMA" (6) and numerous commercial kits have been developed based on this principle. With the preparation of highly purified ferritin it then became possible to develop a conventional radioimmunoassay (RIA) utilizing ^{125}I-labeled ferritin (7). Since RIA and IRMA methodologies suffer from similar disadvantages (e.g., short reagent shelf lives, special requirements and restrictions for handling radioisotopes, radioactive waste disposal problems and the need for sophisticated gamma counting equipment) the development of suitable enzyme immunoassays was welcomed as a great advance in clinical chemistry (8–14).

Although the first enzyme immunoassays developed did not achieve the precision and the sensitivity shared by RIA and IRMA techniques (8, 9, 12), later improvements in assay design enable them to compete with the radiometric assays (10, 11, 13, 14). Ferritin enzyme immunoassays have now become commercially available (15).

An ELISA method has recently been developed by the manufacturer of an IRMA kit; similar antibodies are used in both kits. We have evaluated the quality of this new ELISA.

Materials and Methods

Ferritin assay systems

We used IRMA kits from Behringwerke AG, Marburg, Germany (RIA-gnost® Ferritin) and from Ramco Laboratories, Houston Texas 77098, USA (Fer-iron®) and an ELISA kit from Ramco Laboratories (Spectro Ferritin®) for this study.

RIA-gnost® Ferritin

An evaluation has been reported by *Thornton & Waters* (16). We performed the assay according to the package insert instructions. The sample requirement for a single assay is 100 µl, but to compensate for limited linearity, we used 50 µl. Data reduction is performed by means of a weighted linear regression using a logit-log calculation (17). We utilized a computer program capable of curve-fitting and rejecting those results falling outside the 95% confidence range of the regression line. The kit's expiration date is generally about 7 weeks after delivery.

Fer-iron®

This commercial assay is similar in its design to that described by *Miles* et al. (6).

Depending on he actual procedure used, the required sample volume for a single determination is either 10 or 20 µl. The total minimum incubation time consists of either 4 hours or 90 minutes and for our evaluation we employed the 4 hour procedure. For the calculation of the results the manufacturer recommends the logit-log method, and we performed this calculation with the aid of the computerized weighted logit-log program. The kit has a shelf life of about 12 weeks when stored at 2–8 °C.

Spectro Ferritin®

This kit is identical to the Fer-iron® kit with the exception that the alkaline phosphatase label and the substrate *p*-nitrophenyl phosphate have replaced the radioisotope. We carried out the assay essentially as recommended by the manufacturer. However, the manufacturer points out that the investigator has the option of varying the incubation period as with the Fer-iron® assay. We employed for our study a sample volume of 10 µl and a total incubation time of 4.5 hours. We calculated the results using the same computer searching logit-log program as we employed for the IRMA approach. The kit expires in about 10 months.

Sample handling

All samples are kept frozen at −20 °C until analysis.

Results

Standard curve

Figure 1 represents the mean of 36 standard curves of Spectro Ferritin® obtained in our evaluation. We observed differences between the four lot numbers tested (001, 003, 004 and 005) with lot 003 exhibiting high absorbance values (up to A = 2.000 for the 2000 µg/l standard). This finding was confirmed by the manufacturer and an improvement was then made by using a shorter incubation period for the determination of enzyme activity. Lot 001 frequently showed poor duplication of the 2000 µg/l standard with absorbance differences up to 0.400 A. We observed an improvement with the other lot numbers.

W DE G

Walter de Gruyter
Berlin · New York

K. Fotherby
S. B. Pal
(Editors)

Hormones in Normal and Abnormal Human Tissues

Volume 1
1980. 17 cm x 24 cm. XIV, 658 pages with figures and tables.
Hardcover. DM 145,–; approx. US $69.25
ISBN 3 11 008031 1

Volume 2
1981. 17 cm x 24 cm. XII, 552 pages with figures and tables.
Hardcover. DM 135,–; approx. US $64.50
ISBN 3 11 008541 0

Volume 3
1982. 17 cm x 24 cm. Approx. 500 pages with figures and tables.
Hardcover. Approx. DM 120,–; approx. US $57.25
ISBN 3 11 008616 6

Hormones in Normal and Abnormal Human Tissues is a **three-volume monograph** dealing with the circulating levels, the pathological and therapeutic conditions and the factors controlling the secretion of non-polypeptide, protein and steroid hormones.

An attempt has been made to place emphasis on the concentration of the various hormones in tissues; where they are produced and where they might localize and produce an effect, and how these levels are modified under various circumstances.

M. K. Agarwal
(Editor)

Hormone Antagonists

1982. 17 cm x 24 cm. IX, 734 pages. Numerous illustrations.
Hardcover. DM 180,–; approx. US $85.75
ISBN 3 11 008613 1

This book groups together under one single cover antagonists for those hormones where antagonism has been documented specifically and with a certain degree of certitude. The major emphasis has been delineation of anti-hormone activity at the level of the hormone receptor but other aspects, such as antibody mediated antagonism and inhibition of synthesis, have been included to indicate other possible levels of inhibition of hormone activity. Clinical aspects, too, have been covered where they were documented with certitude.

It is felt that the book represents a major new reference source for years to come. Scientists, medical academicians, and advanced graduate students may use the book as a departing point for further pursuit of their own field. Involved research workers will find the volume of much interest since it provides data not published elsewhere. The book may also be used as a text volume to indicate the diversity and the wealth of information on the subject of hormone antagonism both in the basic research and in clinical medicine. Photo-offset method of publication assures expediency before specialized articles obsolete novelty.

Prices are subject to change without notice.

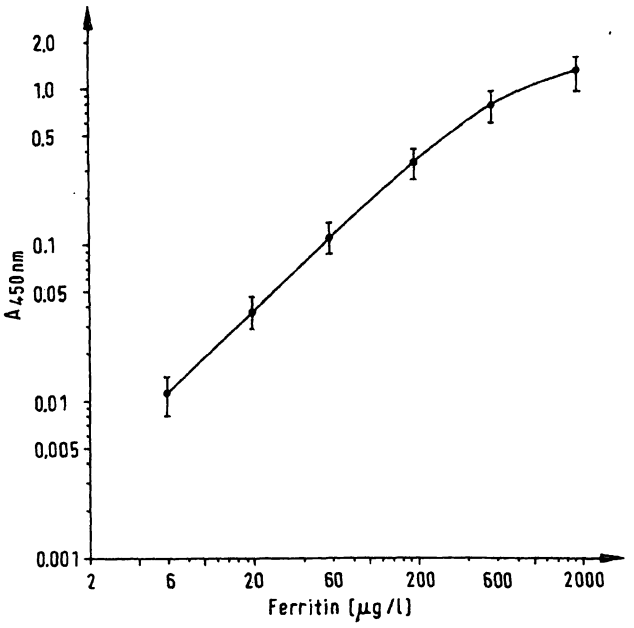

Fig. 1. Standard curve of Spectro Ferritin® (ELISA). This curve is the mean of 36 standard curves ±SD.

Upon storage of the kits the absorbance values decreased gradually (by 5–20% up to the expiry date), but without any detectable influence on the results. Further stability problems were not encountered.

Sensitivity

We evaluated sensitivity in terms of "lowest detection limit", as defined by *Schuurs & Van Weemen* (18). In one assay we evaluated 20 zeros in duplicate and determined the sensitivity by calculating the 95% confidence limits at the zero point of the standard curve.

Based on this approach, we found the sensitivity for the Spectro Ferritin® to be 1.4 µg/l, corresponding to 14 pg per bead, with the assay requiring 10 µl serum. Fer-iron® has a sensitivity of 0.9 µg/l, corresponding to 9 pg per bead.

Precision

We performed precision studies using serum pools containing low, normal and high ferritin levels (tab. 1).

Tab. 1. Precision data.

	Spectro Ferritin®				Fer-iron®				RIA-gnost®			
	n	Mean (µg/l)	±SD	CV (%)	n	Mean (µg/l)	±SD	CV (%)	n	Mean (µg/l)	±SD	CV (%)
	Within-run											
Pool I	20	12.2	1.21	9.9	20	12.1	0.67	5.6	20	42.7	2.30	5.4
Pool II	20	77.3	2.81	3.6	20	86.7	2.11	2.4	20	235	26.8	11.4
Pool III	20	515	37.2	7.2	20	691	32.4	4.7	–	>500	–	–
	Between-run											
Pool I	20	11.9	1.42	11.9	8	13.2	1.41	10.7	10	34.6	4.01	11.6
Pool II	20	85.9	7.66	8.9	10	89.7	10.14	11.3	10	172	9.7	5.7
Pool III	20	618	75.8	13.3	10	720	50.1	6.9	–	>500	–	–
					(9	624	45.8	7.3)[a]				

[a] Values obtained after manual construction of the standard curves and recalculation of the results.

Tab. 2. Differences in the results of Spectro Ferritin® and Fer-iron®.

	Pool I	Pool II	Pool III	
Differences of the means (µg/l)	1.3	3.8	102	(6)[a]
95% confidence interval for the differences (µg/l)[b]	0.1–2.5	−3.6–11.2	54–150	(−40–52)

[a] Values obtained after manual construction of the standard curves and recalculation of the results.
[b] Calculated with use of the *Student*'s t-test (p = 0.05).

With Fer-iron® we obtained a considerable higher mean value for pool serum III than with Spectro Ferritin®. This is a result of the fact that the 600 µg/l standard does not exactly fit the linear regression line of the logit-log calculation method for Fer-iron®. This standard point lies in most cases above the standard curve, causing an overestimation in that area. We have reconstructed the standard curves manually so that standard point 600 µg/l fitted the curves and recalculated the values for serum pool III. This results in a mean ferritin concentration of 624 µg/l instead of 720 µg/l (tab. 2).

Recovery

Recovery studies have been performed by adding ferritin standards to sera with different ferritin concentrations. The recovery study results are expressed as the relative amount of added ferritin found after assay determination. The average recovery was 103 ± 13% (n = 17) for a final concentration range of 28–1950 µg/l.

Linearity

The serum pools containing serially diluted (11-fold) normal and high ferritin levels were also analysed in separate runs. The assay results are listed in table 3.

Dilution of the highest standard (2000 µg/l) showed good linearity and parallelism when compared with the standard curve (fig. 2). Dilution of serum samples does not result in a significant deviation from parallelism in comparison with the standard curve, except in the high dose region (fig. 2). Some serum samples demonstrated good linearity up to 1800 µg/l, whereas others deviated from linearity above 500 µg/l. The "high dose hook" phenomenon, as described by *Miles* et al. (6), was not observed in two serum samples with ferritin concentrations of 25000 and 40000 µg/l (fig. 2). In our search for a possible influence of the high dose hook effect we determined 30 serum samples containing ferritin concentrations ranging between 1000 and 2000 µg/l undiluted and diluted 1:10. Some representive results are given in table 4. In most cases we observed a deviation from linearity with Spectro Ferritin® and with Fer-iron®. However we did not observe a drastic increase of the assay results after dilution, as would be expected if the hook effect were present.

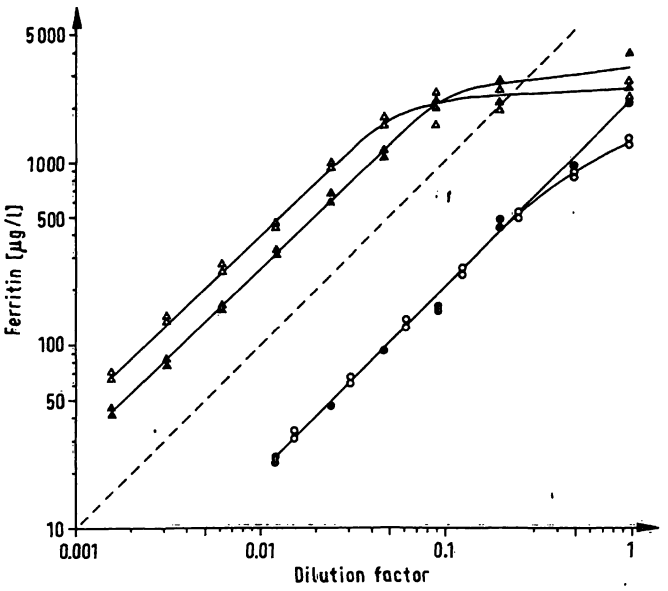

Fig. 2. Linearity of Spectro Ferritin®. Dilution of a high standard and patient sera. Standard, 2000 µg/l (●); serum sampl, 40000 µg/l (△); serum sample, 25000 µg/l (▲); serum sample, 2050 µg/l (○). Dilutions must parallel the dotted line (– –). Values above 2000 µg/l were measured by extrapolating the standard curve.

Tab. 3. Ferritin concentration at dilution.[a]

	Spectro Ferritin®	
	Pool II Mean ± SD (µg/l)	Pool III Mean ± SD (µg/l)
Undiluted	85.9 ± 7.66	618 ± 75.8
11-fold diluted	94.5 ± 14.40	636 ± 68.3
Difference of the means	8.6	18
95% confidence interval for the difference	2.2 – 15.4	−22 – 62
n	20	20
	Fer-iron®	
	Pool II Mean ± SD (µg/l)	Pool III Mean ± SD (µg/l)
Undiluted	89.7 ± 10.14	720 ± 50.1 (624 ± 45.8)[b]
11-fold diluted	109.9 ± 6.05	635 ± 46.0
Difference of the means	20.2	85 (11)
95% confidence interval for the difference	9.7 – 30.7	54 – 116 (−32 – 54)
n	10	10

[a] Values obtained for the diluted samples are corrected for dilution. The confidence intervals are calculated with use of *Student*'s t-test for paired samples at p = 0.05.

[b] Values obtained after manual construction of the standard curves and recalculation of the results.

Tab. 4. Ferritin values (µg/l) at two dilutions.

Spectro Ferritin®		Fer-iron®	
Undiluted	11-fold diluted	Undiluted	11-fold diluted
1118	1694	1684	1540
1520	2475	2367	2684
1114	1419	1656	1430
1374	2453	1716	2761
1867	2827	1386	2486
1472	1827	1080	1848
2029	2882	1279	2827
1739	2563	1508	2409
761	1133	1085	n.d.
≥2000	6360	1924	6567

n.d., not determined.

It is unlikely that the hook effect caused invalid ferritin results below 1000 µg/l, because this would be detected during the sample comparison with the RIA-gnost® assay (the latter requiring sample predilution at a low level).

Interference

Serum samples containing high and low ferritin concentrations and above normal concentrations of possible interfering substances were analysed with the Spectro Ferritin® method.

The following substances do not produce any significant interference: 19 mmol/l triglycerides, 50 g/l human albumin, 100 g/l human γ-globulins, 500 µmol/l bilirubin and 0.05–0.2 mmol/l haemoglobin.

When we compared the ferritin concentration of EDTA-plasma (7.2 mg of EDTA per 5 ml of blood) with that of serum we observed a decrease of 6% in the plasma ($n = 10$; $p < 0.05$). EDTA-plasma also gives 6% lower values compared with serum when assayed with the Fer-iron® kit. However we obtained 1% higher values with EDTA-plasma as compared with serum by using the RIA-gnost® assay. Increasing the EDTA concentrations to 10 g/l blood has no further influence on these results.

Comparison of methods

We determined ferritin levels of 1–2000 µg/l in the sera of 127 healthy persons and patients, using the Spectro Ferritin® and the Fer-iron® kits. We did not predilute the sera. Figure 3 illustrates these results. Due to the wide range of the concentrations found, the logarithm of the concentrations were compared.

The sign test and the *Wilcoxon* matched-pairs test revealed that at a level of $\alpha = 0.05$ the Fer-iron® results are significantly higher than those of Spectro Ferritin® ($p = 0.01$ and $p < 0.0001$ respectively). For concentration ranges of 0–500 µg/l, these tests showed no significant differences between the kits at a level of $\alpha = 0.05$ ($p = 0.25$ and $p = 0.07$ respectively). A sample comparison of the Spectro Ferritin® assay with the RIA-gnost® assay is shown in figure 4.

Reference values

In order to establish reference values for the Spectro Ferritin® assay we obtained 190 samples from healthy individuals aged 18–60 years. Of these

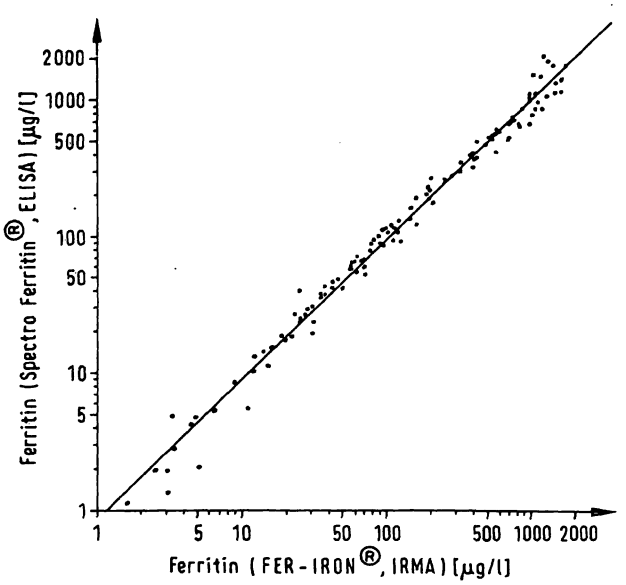

Fig. 3. Comparison of Spectro Ferritin® (ELISA) with Fer-iron® (IRMA). $y = 0.87\ x^{1.01}$, $r = 0.994$, $n = 127$.

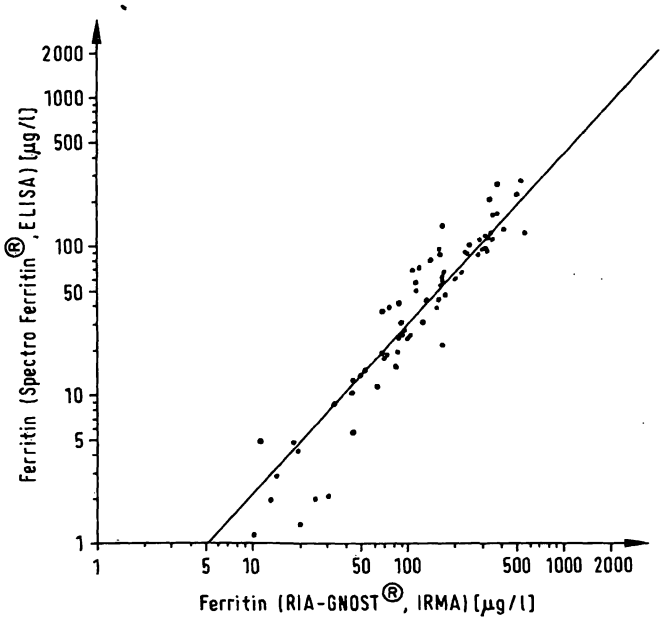

Fig. 4. Comparison of Spectro Ferritin® (ELISA) with RIA-gnost® (IRMA). $y = 0.15\ x^{1.15}$, $r = 0.935$, $n = 76$.

specimens 90 were male and 100 were female. We excluded from this study blood donors or persons who had abnormal levels of serum iron, transferrin or abnormal routine haematological parameters (RBC, Hb, Ht, MCV, MCH, MCHC). The levels found in women were lower than in men and were age dependent (fig. 5). The reference values obtained by the 2.5th to 97.5th percentile estimate are shown in table 5 together with the values obtained with the other kits.

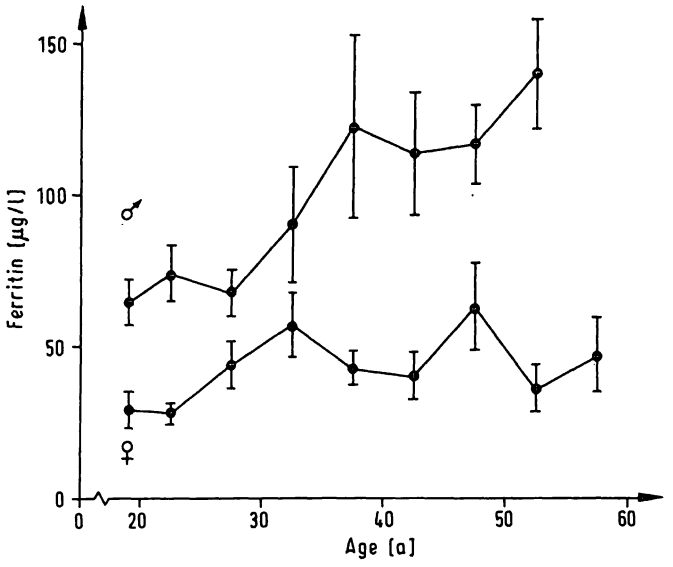

Fig. 5. Age dependency of serum ferritin reference values. Each point represents a 5-year average ±SEM.

Tab. 5. Reference values for serum ferritin.

	Males		
	Geo-metric mean (µg/l)	Range	Source
Spectro Ferritin®	82	30–240	this study
Fer-iron®	84	31–294	package insert
RIA-gnost®	120	30–400	package insert
	Females		
	Geo-metric mean (µg/l)	Range	Source
Spectro Ferritin®	34	10–140	this study
Fer-iron®	29	4–233	package insert
RIA-gnost®	60	30–150	package insert

Discussion

The Spectro Ferritin® assay (ELISA) is just as practicable as the Fer-iron® assay (IRMA). While the Spectro Ferritin® assay has the advantage that no special precautions have to be taken against radioactive contamination, it does necessitate an extra incubation step of 1/2 hour for the determination of the enzyme activity, producing a total incubation time of 4.5 hours. Another disadvantage of the Spectro Ferritin® kit is the cost. The price per bead is about 12% higher than those of Fer-iron® and RIA-gnost®. It is evident that expensive gamma counting equipment is not required. In our hands the data reduction by logit-log transformation applied better to the Spectro Ferritin® assay than to the RIA-gnost® or Fer-iron® systems, even though the logit-log method is advised for the latter assay. Another great advantage of the Spectro Ferritin® assay is the long shelf life (about 10 months) without detectable stability problems. The sensitivity of Spectro Ferritin® (detection limit: 1.4 µg/l or 14 pg/bead) corresponds well with not only those of other ferritin ELISA's (12.5 pg/tube (10) and 10 pg/tube (11)) but also with that of Fer-iron® (detection limit: 0.9 µg/l or 9 pg/bead). *Miles* et al. (6) reported for their IRMA a sensitivity of 29 pg/tube). If we also consider the normal values of ferritin, the detection limit of Spectro Ferritin® seems to be low enough to quantitate ferritin in the serum of patients with iron deficiency.

Based on our precision studies, the Spectro Ferritin® kit works as well as the Fer-iron® method. The within-run precision for Fer-iron® is slightly better than one observes with Spectro Ferritin®, but the between-run precision does not show any great differences in either the normal or low levels (tab. 1). However, we note that the precision of Spectro Ferritin® is better than many other commercial radiometric assays, showing CV's up to 27% between-run (19).

A general problem of IRMA and ELISA procedures has been the "high dose hook effect", first described by *Miles* et al. (6). Since the influence of this effect is assay dependent (20–23), one must evaluate this effect for every ELISA or IRMA system. A number of counter measures have been proposed but in practice the quality of a test kit is crucial. In our evaluation of the Spectro Ferritin® assay we found no high dose hook effect up to 40000 µg/l (fig. 2). If we use an upper linearity limit of 1000 µg/l, the hook effect is very unlikely to occur and in most cases we do not have to estimate our samples at two dilutions. However, we sometimes observed poor duplications of the results at high concentrations, a phenomenon we also noted with the Fer-iron® kit. It is possible that minor differences in antibody coating of the beads are responsible for this effect.

Ferritin determinations on serial dilutions frequently reveal deviations from linearity above 1000 µg/l (fig. 2, tab. 4). This phenomenon can also be observed using the Fer-iron® kit and agrees with the findings of *Li* et al. (22). We question their explanation that the nonlinearity is due to the high dose hook effect,

because the standards and some sera show no deviation up to 2000 µg/l and also because we noted good recoveries up to levels of 1950 µg/l. We recommend diluting and reassaying those serum samples possessing ferritin concentrations above 1000 µg/l.

The influence of EDTA on the ferritin results is inconsistent with reports of other investigators. While some authors found an increase of immunoreactivity (24), or no difference (2), one author found 15–48% lower values (25). We found 6% lower ferritin values in EDTA plasma compared with serum using Spectro Ferritin® and Fer-iron®. RIA-gnost® gave 1% higher values for EDTA plasma. It is known that EDTA is capable of releasing iron from ferritin (24) which may cause a structural change in the ferritin molecule, and result in divergent reactivities with different antibodies.

Recently *Ghielmi* et al. (26) have developed two RIAs an IRMA and two ELISAs for the determination of serum ferritin by using similar antibodies and standard preparations. The ELISAs produced 15% lower values than the IRMA and were less sensitive (a parameter not quantified). Although Spectro Ferritin® is less sensitive than Fer-iron® we did not observe significant differences in the values obtained (tab. 2, 3). A comparison of various samples (fig. 3) demonstrates significantly lower values for Spectro Ferritin® than with Fer-iron®, at levels >500 µg/l, but this difference can be ascribed to the earlier mentioned influence of the logit-log calculation method on the results of Fer-iron®. The ferritin values obtained with RIA-gnost® were about 3 times higher than those obtained with the Ramco kits (fig. 4). The lack of international standardization is responsible for the persistence of these differences, although we should not expect identical values using international standards (26). Near the end of our experimental studies Behringwerke unexpectedly changed the nature of their solid phase antibody causing 20–40% lower ferritin values than observed with earlier kits.

It is our impression that spleen and liver ferritin do not react identically. Sample comparisons of RIA-gnost® (liver standards) with Spectro Ferritin® (spleen standards) show that the regression line possesses a slope of about 1.15 (fig. 4). Spleen standards do not parallel liver standards as assayed with the RIA-gnost® kit. Slight differences when using liver and spleen standards have also been reported in the literature (27, 28).

The normal values we found correspond very well with those observed with Fer-iron® (23) (tab. 5). Our values do not demonstrate extreme concentrations as compared with other ferritin assays (2). The age dependencies correspond very well with earlier findings (29).

We conclude that the substitution of the radioactive label by an enzyme tag does not reduce the reliability of the ferritin immunoassay.

Acknowledgment

We thank Laboratorium Service Benelux B.V., the Netherlands, and Ramco Laboratories, USA, for providing the test kits for this study. We would also like to thank the Red Cross Blood Bank of Rotterdam for supplying many blood samples. We are extremely grateful to Ir. *R. Docter* for the use of his data reduction program and to Prof. Dr. *H. G. van Eijk* for his helpful comments.

References

1. Alfrey, C. P. (1978) CRC Critical Reviews in Clinical Laboratory Sciences *9*, 179–208.
2. Worwood, M. (1979) CRC Critical Reviews in Clinical Laboratory Sciences *10*, 171–204.
3. Kaltwasser, J. P. & Werner, E., eds. (1980) Serumferritin, Methodische und Klinische Aspekte. Springer Verlag, Berlin.
4. Albertini, A., ed. (1980) Radioimmunoassay of Hormones, Proteins and Enzymes, Proceedings of the International Symposium, Gardone Riviera, May 8–10, 1980. Excerpta Medica, Amsterdam, pp. 197–266.
5. Addison, G. M., Beamish, M. R., Hales, C. N., Hodgkin, M., Jacobs, A. & Llewellin, P. (1972) J. Clin. Pathol. *25*, 326–329.
6. Miles, L. E. M., Lipschitz, D. A., Bieber, C. P. & Cook, J. D. (1974) Anal. Biochem. *61*, 209–224.
7. Marcus, D. M. & Zinberg, N. (1975) J. Nat. Cancer Inst. *55*, 791–795.
8. Thériault, L. & Pagé, M. (1977) Clin. Chem. *23*, 2142–2144.
9. Zuyderhoudt, F. M. J., Boers, W., Linthorst, C., Jörning, G. G. A. & Hengeveld, P. (1978) Clin. Chim. Acta *88*, 37–44.
10. Watanabe, N., Niitsu, Y., Ohtsuka, S., Koseki, J., Kohgo, Y., Urushizaki, I., Kato, K. & Ishiwaka, E. (1979) Clin. Chem. *25*, 80–82.
11. Anaokar, S., Garry, P. J. & Standefer, J. C. (1979) Clin. Chem. *25*, 1426–1431.
12. Pagé, M., Thériault, L. & Nilsson, M. (1980) Scand. J. Clin. Lab. Invest. *40*, 641–645.
13. Lee, M. & Burgett, W. (1981) Clin. Chim. Acta *112*, 241–246.
14. Anderson, M. G. & Kelly, A. M. (1981) Clin. Chim. Acta *116*, 405–408.
15. Fortier, R. L., McGrath, W. P. & Thomey, S. L. (1979) Clin. Chem. *25*, 1466–1469.
16. Thornton, J. A. & Waters, H. M. (1980) Med. Lab. Sci. *37*, 275–283.
17. Snedecor, G. W. & Cochran, W. G. (1967) Statistical Methods, The Iowa State University press, Ames, Iowa, USA, 6th ed. pp. 135–171.
18. Schuurs, A. H. W. M. & van Weemen, B. K. (1977) Clin. Chim. Acta *81*, 1–40.
19. Wood, W. G. (1981) J. Clin. Chem. Clin. Biochem. *19*, 947–952.

20. Green, R., Watson, L. R., Saab, G. A. & Crosby, W. H. (1977) Blood *50*, 545–547.
21. Revenant, M. G. & Beaudonnet, A. (1982) Clin. Chem. *28*, 253–254.
22. Li, P. K., Humbert, J. R. & Cheng, C. S. (1978) Clin. Chem. *24*, 1650–1651.
23. Werner, E. & Kaltwasser, J. P. (1980) In: Serumferritin, Methodische und Klinische Aspekte (Kaltwasser, J. P. & Werner, E., eds.) Springer Verlag, Berlin–Heidelberg–New York, pp. 34–55.
24. Porter, F. S. (1974) J. Lab. Clin. Med. *83*, 147–152.
25. Birgegard, G. (1980) Clin. Chim. Acta *103*, 277–285.
26. Ghielmi, S., Pizzoccolo, G., Iacobello, C., Albertini, A. & Arosio, P. (1982) Clin. Chim. Acta *120*, 285–294.
27. Mareschal, J. C., Dublet, B., Wustefeld, C., Charlier, E. & Crichton, R. R. (1981) Clin. Chim. Acta *111*, 99–103.
28. Dunn, C. D. R. & Boden, D. J. (1981) Clin. Chem. *27*, 1280–1283.
29. Finch, C. A., Cook, J. D., Labbe, R. F. & Culala, M. (1977) Blood *50*, 441–447.

Drs. H. A. Assink
Centraal klinisch chemisch Laboratorium
Academisch Ziekenhuis
Dr. Molewaterplein 40
NL-3015 GD Rotterdam